Author's response to reviews

Title: Whole exome sequencing in an Indian family links cerebroretinal microangiopathy with calcifications and cysts (CRMCC) and dextrocardia with a homozygous novel CTC1 and a rare HES7 variation

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Author's response to reviews:

Response to the Reviewer’s comments

We are sincerely thankful to the reviewers for their critical evaluation of our manuscript. This has indeed allowed us to improve our manuscript in terms of scientific expression and the content.

Referee 1

Comments: The aim of the study is to describe a 8 year old boy with a complex phenotype characterized by CRMCC (cerebroretinal microangiopathy with calcifications), dextrocardia and situs inversus, in which a novel homozygous missense variation in the CTC1 gene and a rare known variation in the HES7 gene were detected by targeted resequencing and whole exome sequencing (WES).

As far as concerned the genetic findings, the reported association between a homozygous CTC1 missense mutation with CRMCC phenotype is not new, as widely recently described in several recent studies. Also the association between the HES7 gene and dextrocardia and situs inversus phenotype is known, as reported by Sparrow DB, et al. Am J Med Genet 2013, but it is the first time in which the two genetic defects are described in a same patient.

Despite the study describes the first genetically confirmed case of CRMCC from
India, and an unusual association between two different syndromes usually each other independent, it is of poor interest because it lacks to discuss this association. Overall the paper is conducted with little care: the case is not well described and the language used is not always appropriate.

Reply: We sincerely thank the reviewer for evaluation of our manuscript and providing us the critical comments. We agree with the reviewer that the association of CTC1 and HES7 mutations with the CRMCC and Dextrocardia, respectively, is known and well described. To the best of our knowledge this is the first report to discuss the observation of disease associated variations in both in CTC1 and HES7 in a single patient with combinatorial phenotype of the CRMCC and Dextrocardia. The association of both the mutation in the described patient is explained on the basis of long run of homozygosity in the patient surrounding the CTC1 region which also includes HES7 variation. It is expected that long run of homozygosity can be observed due to very small family (parents and trios). It is also noteworthy, that although parents are non-consanguineously married but still we observed homozygous mutation in the CTC1 and run of homozygosity. This could be possible due to the nature of marriage patterns in India which are commonly endogamous (within the ethnic communities). We have modified the discussion of the manuscript to include the discussion pertaining to the combinatorial association of both the CTC1 and HES7 variation. We have also worked upon the language and improved the clinical case description in particular.

Major revisions:

Comment 1: The reported genetic defect of HES7 gene is a known rare variation in dbSNP so its real pathogenic role it is unclear. The Authors don’t discuss this issue.

Answer: We agree with the reviewer that variation of HES7 is the dbSNP entity but we try to emphasize that the homozygous genotype of this HES7 has never been reported both in public domain date resources (1000Genome project) and our analysis of HES7 variation in ethnicity matched large number of controls chromosomes. We have included in the text the appropriate discussion pertaining to the likely pathogenicity to this and we have also described that one of the novel missense homozygous variation obtained through WES data in this family i.e. variation in the TEK (Tyrosine Kinase) can also play role in the pathogenesis of clinical phenotype through notch signalling together with the HES7. However this is speculative, but the fact is that both the TEK and HES7 variations are rare alleles in the normal population. Now the discussion is described as follows:

“Molecular links of dextrocardia/situs-inversus phenotype in notch signaling target genes

We observed a novel homozygous genotype in the 3’ UTR of HES7. Missense mutations of HES7 have been reported to cause Spondylocostal Dysostosis 4 (SCDO4) [11]. A duplication mutation c.400_409dupAAACCGCCCCC in HES7 has been reported to cause dextrocardia with situs inversus in three out of four individual showing SCOD4 manifestations, suggesting a incomplete penetrance
of left-right patterning defect [12]. In our patient, we did not find any skeletal deformities (abnormal vertebral segmentation, ribs fusions, shortened trunk etc. which are usually reported with SCOD4. The null HES7-mice show disruption of anterior-posterior polarity but no disruption of left-right patterning [12]. Unfortunately, we cannot show direct functional consequence of rs182882481 homozygous variation on HES7, but we speculate the role of this variation on HES7 mRNA stability and its effect on subsequent downstream signaling pathway. It is known that HES7 is a transcriptional repressor of LFNG (Lunatic Fringe) and an autorepressor as well while, LFNG regulates notch signaling through a feedback loop mechanism [13]. Their cyclic gene expression plays an important role in the segmentation presomitic mesoderm (PSM) and 3′UTRs of both the genes are critical determinants of cyclical pattern of their expression by controlling mRNA stability [13]. Both the genes are activated by notch signaling and notch signaling plays an important role in the left-right pattern determination during embryogenesis and periodic somatic segmentation [14,15].

We also observed a missense homozygous variation (p.E103D) through WES data in TEK (Tyrosine kinase, endothelial) (Table 1) which is also the target of notch signaling. However, we observed the same TEK variation in a heterozygous state in one healthy control subject from in-house generated whole exome data set of 35 ethnicity matched controls. TEK a receptor protein with its ligand ANGPT1, promotes vasculogenesis through notch signaling [16]. Notch signaling also regulates cardiac development, septal development and coronary vascular development [17]. Cardiac septal abnormalities, like atrial septal defect, ventricular septal defect and coronary artery fistula have been reported in a few cases of CRMCC harboring compound heterozygous mutations in CTC1 [4], this is also suggestive of the role of CTC1 in notch signaling. Therefore it is possible that the overall complex association of CRMCC and dextrocardia/situs-inversus might be the resultant of multiple genetic factors comprising CTC1, HES7 and TEK and their cross talk through notch signaling deregulation. However, It is also possible that even H484P change of CTC1 may be the sole determinant of the pathophenotype in our patient.

Comment 2: The CTC1 and HES7 genes belong to the family of transcription factors regulating Notch signaling pathway. This data should be discussed as the possible clinical consequences of this.

Answers: We have now incorporated the discussion pertaining to the role of notch signaling as mentioned above.

Minor revisions:

In all the test, in figure legend check and add the term gene/genes after the gene symbol. Check that gene symbol CTC1 in all the text, in figure Legend and in references is written in italicus. Many typewrite error (space, full stop, commas, etc.) are present.

Answer: We thank the reviewer for noting errors. We have corrected all the errors and typos.

Abstract:
Comment 1: Line 2: the terms “Cerebroretinal microangiopathy with calcifications and cysts (CRMCC)” and “Coats plus syndrome” are used as synonymous. It is not correct, and the Authors should preferably use the term CRMMC than Coats plus syndrome”. This problem is present in many part of the text.

Answer: As per the literature survey at many instances CRMCC and Coats plus syndrome have been used as the synonym even in the description provided in OMIM/NCBI. Even in the first two papers one published in Nature genetics used the term Coats plus syndrome and another published in AHJG used CRMCC terminology. Nevertheless to avoid confusion we have kept only one terminology for the phenotype i.e. CRMCC.

Comment 2: Line 9: the sentence “Targeted sequencing of CTC1…conducted” should be corrected in “Targeted sequencing of the CTC1 gene…were conducted”

Answer: We have done the correction.

Comment 3: Line 14: the sentence “HES7 was identified as a plausible…” should be corrected in “HES7 was were identified as plausible…”

Answer: We have made the correction.

Background:

Comment 4: Page 5, Line 13: explain the sentence: “In all diseases…central phenotype”. It is uncorrected because in CRMCC phenotype DC and bone marrow failure are not major criteria.

Answer: We thank the reviewer for the suggestion. With this sentence we wanted to convey that among majority of the cases with genetic defect in telomere length maintenance complex, there are certain features which are overlapping. We have refined the sentence which now read as “In all of these diseases, some features of DC like sparse/gray hair, nail dystrophy and bone marrow failure appear as the overlapping phenotype.”

Comment 5: Page 5, Line 17: The sentence “overlap …till date)” should be better explained with a major attention to the proper terminology to describe the clinical continuum observed among these pathologies.

Answers: We have corrected the sentence to bring the clarity which now read as “CRMCC cases also show phenotypic overlap with Revesz syndrome (exudative retinopathy, intracranial calcification, bone marrow failure and shortened telomeres) as well as Labrune syndrome (LCC; leukoencephalopathy, intracranial calcifications and cysts)”

Clinical presentation

Comment: Page 6, Line 16: “Coats disease of the eyes” edit in The eyes present ….compatible with the diagnosis of Coats Disease.

Answer: We thank the reviewer for the suggestion and we have modified the sentence as “his eyes manifested retinal telangiectasia and exudates compatible with the diagnosis of the Coats Disease”
Referee 2
Reviewer's report:

This is a very nice paper. The authors should be congratulated on submitting a well written and illustrated report. The clinical diagnosis and description of phenotype seems secure and well described. My main concern is the pathogenicity of the reported CTC1 variant. The authors provide good evidence in favour of causation (the rarity of the variant and predicted pathogenicity in-silico). However, the EVS and 1000 genomes databases do not in my opinion include enough 'controls' from the same ethnic background as the family described here. As such I recommend that the authors screen a larger number of ethnically matched controls. I would also encourage the authors to comment on the fact that this is the only homozygous CTC1 variant identified in a Coats plus (CP) patient so far, and speculate as to why this might be. I also recommend that the authors comment in the conclusion section on the possible association of dextrocardia with CP. At this point and with the recommended changes below I believe that this paper could be accepted. Given that this disease is so rare any new descriptions in the literature should be welcomed, and in particular cases such as these which may expand the phenotypic spectrum.

Response: We are extremely thankful to the reviewer for the appreciating comments on our work and the manuscript. We have provided the description below which explains and covers all the points raised by the reviewer.

Major Compulsory revisions

Comment1. Screen a larger number of ethnically matched control samples.
Reply: We agree with the reviewer that to establish the rarity of the variation, large no. of ethnicity matched controls should be screened. We have adopted the suggestion and included additional ethnicity matched controls which were available in our DNA repository and CTC1 variation screening was conducted in 740 chromosomes (370 healthy controls). The HES7 variation screening was conducted in 782 chromosomes (391 controls). The screening confirms the absence of CTC1 variation among all the controls analyzed.

2. Comment: on the fact that this is the only homozygous CTC1 variant identified in a case of CP, suggest reasons why
Reply: We have incorporated the appropriate points emphasizing that all previously reported CTC1 variations were observed in compound heterozygosity where one variation essentially a protein truncating change. The earlier described families in the literature reportedly had non-consanguineous marriage pattern. In our present case too, even parents have not reported consanguinity but generally in India among large population ethnic groups though, consanguineous marriages are uncommon but mostly endogamous marriage (marriages within cast group) patterns are followed.

Minor Essential Revisions

Comment1. In Figure 1 (V) image and legend, please clarify which parental sequence trace is shown. Carrier parent is not clear enough; please state either
mother or father.

Reply: Figure 1, V shows sanger sequencing based validation of HES7 variation. Both the parents of the family had heterozygous status of the variation. In order to avoid repetition, we had shown sequence trace of only one parent that is mother.

Comment 2. Clarify/comment on why homozygosity mapping was carried out in a non-consanguineous family.

Reply: The parents were non-consanguineously married but, due to the observed homozygous p.H484P (CTC1) in targeted sequencing of CTC1, we anticipated a region of extended homozygosity around which could contain some additional pathogenic variation which can provide the basis of association of dextrocardia phenotype. The co-occurrence of dextrocardia with CRMCC was found to have possible association with HES7 and CTC1 in the identified homozygous stretch of 3Mbp on chr17.

Comment 3. In Results and Discussion at the end of paragraph two, it would be more accurate to say that the observation of shortened telomere length in the DNA of affected individuals supports (rather than confirms) the speculation of conformational change and reduced capacity for binding to telomeric ends. I do not believe that this experiment alone confirms pathogenicity of the variant described.

Reply: We agree with the reviewer that finding shortened telomere length is the only supportive evidence rather confirmatory. We have modified the sentence.

Comment 4. In Results and Discussion at the end of paragraph one, please correct the figure reference. The text refers to the CTC1 variation but Fig1. V shows traces for the HES7 variation.

Reply: We are thankful to the reviewer for the astute observation. We have corrected the figure reference.

Comment 5. In Abstract Results section, please correct the spelling of ‘leukocytes’.

Reply: We have corrected the spelling mistake.

Discretionary Revisions

Comment 1. Minor changes to text which are highlighted with comments on the attached pdf

We are thankful for noting those errors and providing the suggestions. We have corrected each one appropriately.